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THE COMPLEX REGULATION OF RECEPTOR-COUPLED G-PROTEINS

MARTIN RODBELL

Signal Transduction Section, Laboratory Molecular and Cellular Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, U.S.A.

INTRODUCTION

GTP binding proteins (G proteins) play a crucial role in transmembrane signalling (1, 2) Characterized as heterotrimeric structures, G proteins consist of at least 21 different GTP-binding units (α-subunits), five β-subunits and six γ-subunits (3). They couple with more than 300 different types of receptors, including the complex array of olfactory receptors (4). Typified by having seven membrane spanning domains, functional coupling of these receptors requires all three subunits of G-proteins; acylation of the α- and γ-subunits with fatty acyl- and isoprenyl-groups plays a role in membrane attachment and the coupling process (5, 6).

Still unresolved are the topological organization of G-proteins, receptors and the affected signalling devices (phosphodiesterases, phospholipases, adenylate cyclase, ion channels, etc.) in the surface membrane and how these components are linked during the process of signal transduction. Two prominent models (2, 7) invoke the concept of receptor mobility as a basis for explaining the accumulated evidence that a single receptor can activate several G proteins. In these theories, binding of agonists to the receptors promotes coupling with G proteins. Although freely-mobile receptors can be detected, there is abundant evidence for pre-coupled receptors (8–13), for interactions of receptor-coupled G-proteins with the cytoskeletal network (14–22), and there is evidence for clustered aggregates of receptors and G-proteins (23). Several G protein-regulated effectors, including adenylyl cyclase and ion channels, appear to be associated with actin, actin-binding proteins, or other types of cytoskeletal proteins (24–29). These findings imply organized, immobile structures somehow associated with the intracellular cytoskeletal matrix.

Faced with the probability that receptor-coupled G protein systems and their effectors are not mobile structures, how can each receptor-regulated system be compartmentalized, essentially immobile within the plasma membrane, yet the signals are produced in rapidly (fractions of seconds) reversible fashion? What

process or structure brings about such rapid, expeditious communication between components?

OLIGOMERIC STRUCTURE OF G-PROTEINS

The 'disaggregation' theory of signal transduction (29), with recent modifications (30), supplies some of the potential answers. In the original concept, based on the powerful technique of target irradiation analysis (31), receptors assemble stoichiometrically with G proteins as large multimeric structures ($>10^6$ Da) which, when activated by hormones, GTP, and Mg^{2+}, release 'monomers' of GTP-binding proteins; the latter, depending on the type of receptor and G-protein, causes either stimulation or inhibition of effectors such as adenylyl cyclase. From its inception, the model incorporated the notion that receptors and its GTP-binding protein couple stoichiometrically. However, accumulative evidence indicates that, with few exceptions (rhodospin coupled to transducin, for example), heterotrimeric G-proteins exist far in excess (10–100 times) of receptors in the cell membrane. Accordingly, it can be deduced that the functional mass given by irradiation analysis is comprised of multimeric structures of G proteins. With this assumption, for example, the functional mass of Gs coupled to the glucagon receptor, as determined by irradiation analysis, is ≈800 kDa (32). Given the known mass of Gs (90–95 kDa), a multimer of Gs coupled to the glucagon receptor consists of 8–10 monomer (heterotrimers) units; i.e. one receptor activates a number of monomers in the attached multimer, consistent with the catalytic actions of receptors. Supportive biochemical evidence for multimeric structures stems from a series of studies using octylglucoside or digitonin to extract G proteins from brain and liver membranes (30, 33, 34). G proteins extracted with these detergents display hydrodynamic properties suggestive of structures that are considerably larger than observed in cholate and lubrol, the detergents normally used for extraction and purification of heterotrimeric G proteins. Important structural evidence that G proteins are multimeric in their attachment to biological membranes is that all of the endogenous G-protein types in rat brain synaptoneurosomes (Gs, Gi, Go, Gq) can be cross-linked to yield structures comparable in size to cross-linked, multimeric tubulin associated with the same membranes (35). Although the β-subunits (and presumably the tightly-coupled γ-subunits) are included in the cross-linked material, noticeable differences in the sizes and distribution of sizes were observed (35). The basis of these differences is unknown, but could be interpreted as evidence that the multimers are not solely constructed of heterotrimers.

Evidence obtained with pre-activated adenylyl cyclase in brain and turkey erythrocyte membranes indicates that heterotrimeric Gs is attached to the enzyme (7). Additionally glucagon, combined with GTPγS and Mg^{2+}, activates

in isolated liver membranes a large Gs-containing structure yielding monomeric Gs; interestingly, the large structure is particularly sensitive to the ADP-ribosylating actions of cholera toxin (34). Similarly, activation of several species of G-proteins in brain synaptoneurosomes by GTPγS and Mg^{2+} also produces monomers from larger structures, the relative effectiveness of the nucleotide being Gi=Go>>Gs>Gq (30). These findings suggest that multimers of G-proteins are the principal structures associated with receptors and that the products of hormonal-activation are monomers (heterotrimeric G-proteins).

RECEPTOR ACTIVATION OF G-PROTEINS

Illustrated in Figure 1 is a hypothetical reaction scheme (adapted from (1) depicting interactions between receptor and multimeric Gs. The fundamental premise in this scheme is that the oligomeric chain of G-proteins is an asymmetric structure in constant state of flux depending on the distribution of bound GTP or GDP at the two ends of the structure. Depicted is the release of a GTP-bound monomer from one end and the coupling of GDP-bound monomer at the opposite end. This distribution is governed by the receptor's ability to induce, after activation by a hormone, an exchange between bound GDP and free GTP. During this exchange reaction, a transition state of the receptor-G-protein complex takes place in which the receptor and nucleotide-free G-protein form a tight complex, the bound hormone having a very low dissociation rate (as first seen for glucagon binding to its hepatic receptor (36). Fundamental to the overall reaction scheme is the ability of the hormone-occupied receptor to move along the oligomeric chain of G-proteins in an oscillatory fashion. The frequency and strength of attachment between receptor and G-protein and the degree of activation of G to its GTP-bound state is dictated by the hormone-induced exchange reaction, the resultant transition state, and the subsequent filling of the monomers with GTP; GTP ultimately induces the dynamic instability of the oligomer and release of GTP-bound monomer for action on effectors. This reaction scheme provides in essence the means for receptors to move in an oscillatory fashion along the multimeric chain in a fashion similar to the well-known nucleotide-dependent movement of myosin along the chain of F-actin, an oligomeric counterpart to oligomeric G-proteins. It has been reported that GDP is more effective than GTP in reducing receptor affinity for hormone (37). Hence, during its excursion along the chain, the receptor displays both high and low affinity states for its agonist; these states reflect the strong and weak coupling states between receptor and G-protein which, in turn, reflect the absence of bound nucleotide (strong coupling), and the binding of either GDP or GTP (weak coupling), to the G-protein subsequent to the agonist-induced exchange reaction. Potentially, depending on the relative concentrations of GTP and GDP within the vicinity of the transduction process, each of the monomers can be occupied and activated by GTP. It should be emphasized that the only 'high-affinity' state

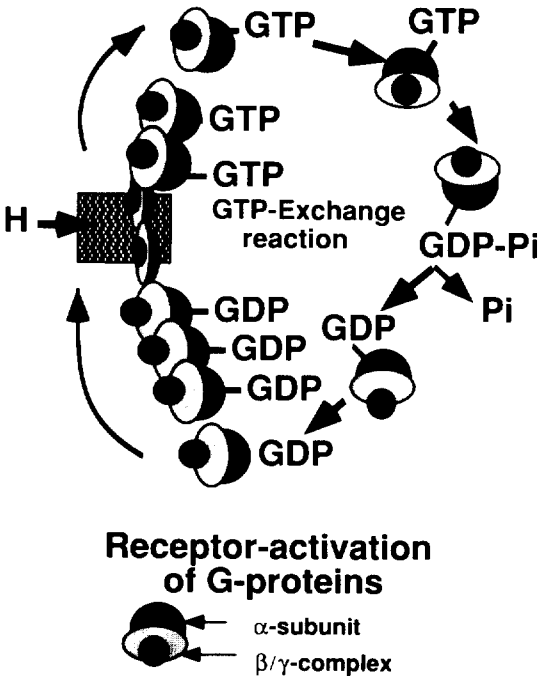

FIG. 1. Model for receptor activation of multimeric G-proteins. All components remain bound to the surface membrane during the entire process. G proteins contained in the multimer are depicted in three states: some with bound GDP; some unoccupied by nucleotide (tightly interacted with high-affinity, hormone (H)-occupied receptor), and some occupied by GTP. Coupling of receptor to the chain is stochastic with relative coupling efficiency of each monomer being a function of the amount and type of nucleotide bound: unbound>>GTP>GDP. Loose and tight coupling of the receptor during its transit along the chain is the result of the differing affinity states of the G-proteins (monomers) in the chain.The nucleotide uptake or exchange reaction occurs at each monomer unit irrespective of loose or tight coupling. As depicted, the most efficacious activation occurs with the high affinity, tightly-coupled form of the receptor. Release of monomers occupied by GTP from multimers is depicted in 'quantal' fashion from one end of the multimer. In this scheme hydrolysis of GTP by GTPase and release of PI are factors that control cycling between monomers and multimers. See text for other details.

of the receptor during the process of G-protein activation is the transition-coupled state induced by the exchange reaction. Although transient, this state is fundamental to the action of agonists.

An interesting outgrowth of this concept is the possibility of explaining the growing evidence that a single receptor not only catalytically activates several of the same species of G-proteins but also more than one species of G-proteins (38–40). This phenomenon can be explained if the multimers contain two or more types of G-proteins, each type regulating different types of effectors.

DYNAMIC ROLE OF GTPASE

A dominant theme in signalling mechanisms is that binding of GTP induces action whereas hydrolysis of GTP leads to inaction. This 'turn-on, turn-off' mechanism remains a dominant theme with all types of G-proteins both heterotrimeric (coupled to receptors) and the small molecular weight G-proteins represented by the Ras family. This idea, however, seems an oversimplification of what appears to be a more complex role of GTPase. For example, with many of the small G-proteins, GTPase activity is stimulated by the association with GTPase activating proteins (41). In the case of heterotrimeric G-proteins, association of Gq, a G protein that activates phospholipase C_β, brings about a marked increase in GTPase activity of Gq_α (42). Based on these examples, GTPase activity, though intrinsic to the α-subunits of heterotrimeric G-proteins, is manifested primarily when these proteins unite with their effectors. The low level or absence of intrinsic GTPase activity observed generally with purified G-proteins agrees with this assertion. Furthermore, modeling of the kinetics of Mg-and Gpp(NH)p-activation of the glucagon-sensitive adenylyl cyclase system in liver membranes gave rise to a transition-state model in which GTPase activity of the enzyme system (E) is only revealed when the system is in its high activity state (E") (43). The transition state model is consistent with the proposition that the effector adenylyl cyclase stimulates GTPase activity of $G\alpha_s$, the cyclase stimulatory α-protein, in the formation of state E". In this model, the role of the GTPase is partly to maintain the signalling system in a dynamic state.

A possible additional role of GTPase is utilization of the energy of hydrolysis to promote a configurational/structural change in the relationship between the α and βγ subunits of G-proteins and the effectors (adenylyl cyclases, phospholipases, channels, etc.) to which these units are attached. The basis of this hypothesis stems from the different regulatory effects of the α and βγ units exemplified from studies of the large family of adenylyl cyclase systems found in different cell types or tissues (44, 45). Some of these systems are inhibited by βγ, others are stimulated, and in some cases βγ does not affect cyclase activity (45). One means of explaining the diverse effects of βγ is to posit that each cyclase molecule has distinct domains for interacting with the α and βγ subunits and that these domains are different for each type of cyclase. Hence, when Gs interacts with a given cyclase to produce a transition state (E′ in Figure 2), this state induces the splitting of GTP to GDP and Pi, resulting in dissociation of α from βγ in coordination with the domains on the cyclase 'template' that bind these subunits. In this manner, for example, the same Gs when interacted with a cyclase having a βγ-inhibitory domain results in decreased cyclase activity relative to a different cyclase having a βγ-stimulatory domain. In essence, the energetics of GTP-hydrolysis provides for a variety of regulatory expressions of adenylyl cyclase (or other signalling

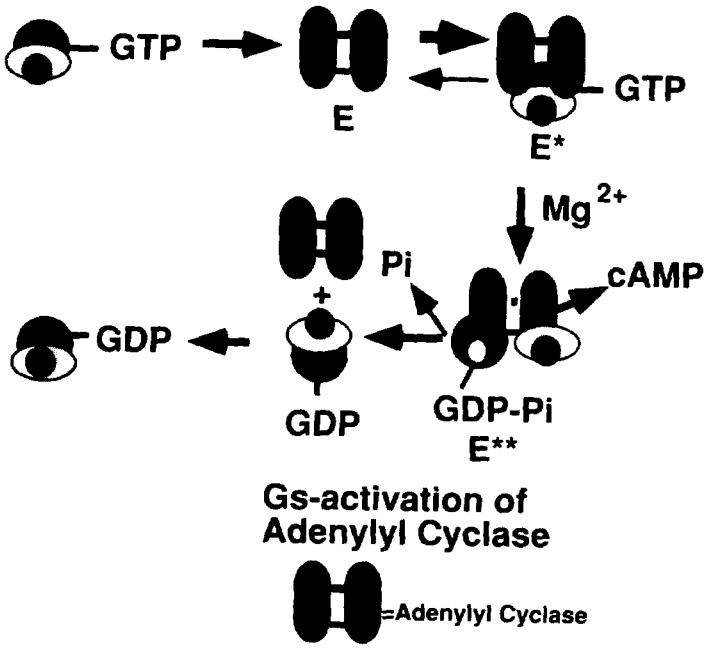

FIG. 2. Activation of adenylyl cyclase by Gs, the stimulatory GTP-binding protein. A GTP-bound heterotrimeric Gs released from the multimeric structure in Figure 2 during hormonal activation interacts with adenylyl cyclase in its basal state (E) to form the transition state (E*). Mg^{2+} is required for hydrolysis of GTP to GDP + Pi and induces separation of bound-heterotrimer into a different configuration of $G\alpha_s$ and $G\beta/\gamma$, each acting at separate sites on the putative 'cassettes' of the enzyme (44). This form of the enzyme (E**) is a high-activity state resulting in increased production of cyclic AMP, rapid release of Pi and concomitant release of Gs containing bound GDP. Gs re-combines with the multimeric structure depicted in Figure 1 to complete the reaction cycle.

systems that similarly contain multiple domains selective for the α and $\beta\gamma$ subunits of G-proteins).

Illustrated in Figure 2 is adenylyl cyclase with two domains in its basal, quiescent state (E), a transition state (E*) when interacted with GTP-bound Gs, and E**, a GTPase-activated state (depicted as two interconnected components each associated with either α or $\beta\gamma$ subunits). Mg ions play a critical role in the transition presumably by serving as a complex with GTP for the catalysis to GDP + Pi. It is suggested that release of Pi from its binding site on the α-subunit allows the latter with GDP-bound to re-associate with $\beta\gamma$. GDP-bound Gs disassociates from adenylyl cyclase and subsequently re-unites with the oligomeric structure of G-proteins at the opposite end from which the GTP-activated Gs dissociated (Fig. 1) in response to the concerted activating effects of hormone and GTP.

SUMMARY

Heterotrimeric G-proteins are associated with the cytoplasmic surface of the cell membrane as oligomeric structures. The oligomeric structures were deduced from a variety of studies including target (irradiation) analysis, hydrodynamic evaluation of detergent extracted material, and cross-linking of G-proteins in their membrane environment. From the functional mass determined by target analysis, it was estimated that one receptor (for glucagon) is associated with 8–10 units of Gs, the heterotrimeric G-protein that stimulates adenylyl cyclase. It is proposed that the receptor associates with each monomer of the chain via weak and strong binding forces that are dictated according to whether either GTP or GDP is bound to the α-subunits (weak forces) or, due to the hormone-induced release of the nucleotides during the exchange reaction, these subunits become transiently devoid of nucleotides (strong forces). The hormone-induced changes in type and degree of nucleotide binding allow for movement of the receptor along the oligomeric chain and filling of the nucleotide binding sites with the activating nucleotide, GTP. In this manner, the receptor catalytically activates Gs. It is suggested that the dynamic instability of the oligomeric chain produced by the asymmetric distribution of GTP and GDP along the chain results in release of a GTP-monomer from one end and association of a GDP-monomer at the opposite end. Adenylyl cyclase associates with the released GTP-monomer inducing a transient state of the coupled proteins. In a Mg-dependent fashion, hydrolysis of GTP occurs resulting in re-organization of the coupled proteins such that α and βγ interact with distinct domains of the cyclase molecule. The final state of the coupled process determines the degree of cyclase activity. Release of Pi from its binding site restores association of α and βγ to the GDP-bound form of the heterotrimer. The latter associates with the oligomeric structure of G-proteins to complete the cycle of events in the overall process of hormonal activation of the system.

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